

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Claim 1 (currently amended) A method for classifying and counting leukocytes comprising the steps of:

(1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other:

(a) a first fluorescence-labeled antibody which binds specifically to leukocytes,

(b) a second fluorescence-labeled antibody which binds to at least one kind of neutrophilic cells, and

(c) a third fluorescence-labeled antibody which binds to at least one kind of immature granulocytic cells,

in order to stain the leucocytic cells in the hematological sample;

(2) removing erythrocytes from the hematological sample;

(3) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

(4) classifying granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(5) distinguishing eosinophils and neutrophilic cells in the granulocytic cells obtained in step (4) on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody;

(6) classifying the neutrophilic cells obtained in step [(4)] (5) into groups different in degree of maturity on the basis of the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody; and

(7) counting the number of cells in each of the groups.

Claim 2 (currently amended): A method according to claim 1, wherein in step [(3)] (4), a group of all leukocytic cells is defined and counted on the basis of the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody in addition to the granulocytic cells obtained in step [(3)] (4), and in step [(5)] (6), the ratio of the number of the neutrophilic cells obtained in step [(4)] (5) different in degree of maturity with respect to the number of all the leukocytic cells is calculated.

Claim 3 (original): The method according to claim 1, wherein the first fluorescence-labeled antibody comprises an anti-CD45 antibody.

Claim 4 (original): The method according to claim 1, wherein the second fluorescence-labeled antibody comprises an antibody selected from the group consisting of an anti-CD11b antibody, an anti-CD16 antibody, an anti-CD66b antibody and an anti-CD66c

antibody, and the third fluorescence-labeled antibody comprises an antibody selected from the same group but different from the antibody of the second fluorescence-labeled antibody.

Claims 5-6 (canceled).

Claim 7 (original): The method according to claim 1, wherein the scattered light measured is side scattered light.

Claim 8 (original): The method according to claim 1, wherein the fluorescent dyes are selected from the group consisting of fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Texas Red, PE-CY5 and peridinin chlorophyll protein (PerCP).

Claim 9 (original): The method according to claim 7, wherein the fluorescent dyes of the first, second and third fluorescence-labeled antibodies for emitting distinguishable fluorescences comprise a combination of FITC, PE and PE-CY5 or a combination of FITC, PE and PerCP.

Claim 10 (original) The method according to claim 1, wherein the hematological sample is a sample of peripheral blood, bone marrow fluid or urine of a mammal.

Claim 11 (currently amended): The method according to claim 1, wherein ~~in step~~ (1), the leukocytic cells are fluorescence-stained after the erythrocytes are removed from the hematological sample.

Claim 12 (currently amended): The method according to claim 1 that in the step [(4)] (5), a two-dimensional scattergram is produced from the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody, and the eosinophils and the group of neutrophilic cells in the granulocytic cells obtained in step [(3)] (4) are distinguished on the two-dimensional scattergram.

Claim 13 (currently amended): The method according to claim 1 that in the step [(3)] (4), a two-dimensional scattergram is produced from the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody, and the granulocytic cells obtained in step [(3)] (4) are distinguished on the two-dimensional scattergram.

Claim 14 (currently amended): The method according to claim 1 that in the step [(5)] (6), a two-dimensional scattergram is produced from the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody, and the neutrophilic cells are classified according to degrees of maturity on the two-dimensional scattergram.

Claim 15 (canceled).

Claim 16 (new): A method for classifying and counting leukocytes comprising the steps of:

(1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other:

(a) a first fluorescence-labeled antibody which binds specifically to leukocytes,

(b) a second fluorescence-labeled antibody which binds to at least one kind of neutrophilic cells, and

(c) a third fluorescence-labeled antibody which binds to at least one kind of immature granulocytic cells,

in order to stain the leucocytic cells in the hematological sample;

(2) removing erythrocytes from the hematological sample;

(3) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

(4) classifying granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(5) distinguishing eosinophils and neutrophilic cells in the granulocytic cells obtained in step (4) on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody;

(6) classifying the neutrophilic cells obtained in step (5) into groups different in degree of maturity on the basis of the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody; and

(7) counting the number of cells in each of the groups;

wherein the second and third fluorescence-labeled antibodies comprise any combination of an anti-CD16 antibody with an anti-CD11b antibody, an anti-CD16 antibody with an anti-CD66b antibody, an anti-CD16 antibody with an anti-CD66c antibody, an anti-CD11b antibody with an anti-CD66b antibody, and an anti-CD11b antibody with an anti-CD66c antibody.

Claim 17 (new): The method according to claim 16, wherein the second and third fluorescence-labeled antibodies comprise the anti-CD16 antibody and the anti-CD11b antibody.

Claim 18 (new): A method for classifying and counting leukocytes comprising the steps of:

(1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other:

(a) a first fluorescence-labeled antibody which binds specifically to leukocytes,

(b) a second fluorescence-labeled antibody which binds to at least one kind of neutrophilic cells, and

(c) a third fluorescence-labeled antibody which binds to at least one kind of immature granulocytic cells,

in order to stain the leucocytic cells in the hematological sample;

(2) after the adding step (1), removing erythrocytes from the hematological sample;

(3) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

(4) classifying granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(5) distinguishing eosinophils and neutrophilic cells in the granulocytic cells obtained in step (4) on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody;

(6) classifying the neutrophilic cells obtained in step (5) into groups different in degree of maturity on the basis of the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody; and

(7) counting the number of cells in each of the groups.